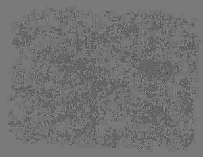
DETERMINATION OF ARSENIC IN URINE



March 1977

Ministry of the Environment
Ontario

The Honourable
Harry C. Parrott, D.D.S.,
Minister

Graham W. S. Scott,
Deputy Minister

DETERMINATION OF ARSENIC IN URINE

BY

PREM N. VIJAN

MARCH 31, 1977

REPORT ON PROJECT PNV 7502

Arsenical minerals have been mined and processed in the past around the Red Lake district in Ontario. It was suspected that arsenic, considered to be a toxic material, may be seeping into the water systems causing wide spread contamination. A few instances of arsenic pollution in Federally administered areas have been cited and commented on by the news media thereby arousing the public awareness of the problem. Ontario Ministry of Health wanted to know the concentrations of arsenic in the urine specimens of the native population of Red Lake area. Since the Ministry's own laboratories did not have a reliable and sensitive method for determining arsenic, our laboratory was approached to provide this service. The Air Quality Laboratory had developed a semi-automated method for determining arsenic in air particulates, vegetation and soil which had proved to be quite accurate and sensitive. The method was based on the conversion of arsenic into arsine gas and the vapour phase atomization of the arsenic in a heated quartz cell and measurement of atomic absorption signal (1,2). The method was far superior than the silver diethyl dithiocarbamate colorimetric method which is considered as a standard method for trace concentrations of arsenic and has been adapted by AOAC.

Dr. G. T. Stopps in his memo dated February 28, 1975, requested that the Air Quality Laboratory analyse

...2

approximately 400 urine samples for arsenic. Since the method was already being used for analysis of air particulates, vegetation and soil, no major problem was foreseen and the request was accepted. A high priority was placed on the job and the Project Group was given two weeks to investigate, briefly, the application of the automated method to urine samples. A limited effort at literature search did not show up any previously published work on the use of hydride-AA method for urine analysis. J. G. Lamberton et al[3] have measured arsenic in urine by drying 20 ml urine sample in presence of 3 g magnesium oxide in an oven and then ashing the dried residue with added magnesium nitrate at 550°C in a furnace. The ashed material is dissolved in hydrochloric acid, arsine is generated in a home-made generator and absorbed in a 0.5% silver diethyl dithiocarbamate in pyridine as per recommended AOAC method. Lamberton et als' paper contains other useful references on the subject. This method is designed to be a fast field method using portable fume hood, but it is too involved, time consuming and complicated. The detection limit of the method based on 20 ml sample is 0.03 ppm.

EXPERIMENTAL:

The use of nitric acid-perchloric acid mixture was considered appropriate for wet ashing of urine samples, based on our work on vegetation material[2]. It is very

...3

effective in converting all the organically bound arsenic into inorganic form and has been used by other workers (see cross references[3]).

The following experimental approaches were tried to test the suitability of the method for urine analysis:

a) The loss, if any, due to drying before wet ashing.

b) Wet ashing urine with 4:1 nitric:perchloric acid mixture and fuming nitric mixture without previous drying of urine.

c) Study of optimum volume of urine sample and that of the decomposition acid mixture.

d) Recovery of added inorganic arsenic to normal urine samples. (No organic compounds of As were available in the laboratory for this study).

e) Replicate analysis of a few samples to establish the precision and accuracy of the method.

The conclusions drawn from these experiments are listed under "The Results and Discussions". The analytical system used together with the reagents and apparatus are the same as in reference 2. A diagram of the analytical system is shown in figure 1.

...4

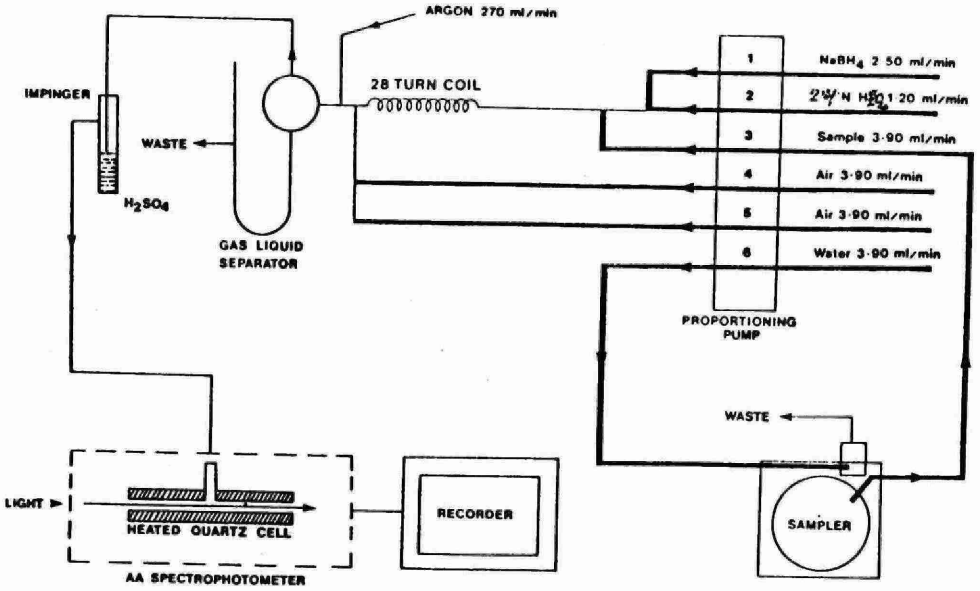


Figure 1. AutoAnalyzer-AAS system for Arsenic

PROCEDURE:

Pipet 2.0 ml urine sample into an 18 x 150 ml test tube calibrated at 10 ml and held in a 40 hole aluminum heating block. Add 2 ml 4:1 nitric-perchloric acid mixture and mix. A reagent blank and three standards consisting of 0.05, 0.10, and 0.20 ml 1 ppm arsenic solution are included in a set of 36 samples to be digested. Every 4th sample is run in duplicate and every 11th sample is spiked with 0.1 ug of arsenic. The loaded aluminum block is transferred to a hot plate and heated to a surface temperature of approximately 120°C. The block may be left overnight on the hot plate. An approx.

...5

1 ml of clear, white digestate is obtained at the end of the digestion period. Allow the contents of the test tubes to cool to room temperature and make the volume to 10 ml mark with distilled water. Seal the test tubes with parafilm and mix the contents. Run the prepared solutions through the analysis system and read the arsenic concentrations from the standard curve.

ug/l arsenic in urine = 5 x ng As/ml.

RESULTS AND DISCUSSION

For the determination of very low concentrations of arsenic present in normal urine samples, it is very important that the sample treatment procedure should be well organized and involve minimum handling. The use of 18 x 150 ml calibrated test tubes for digestion of batches of 40 samples places limitation on the sample volume that can be conveniently handled. An effort was made to oven dry the samples before digestion. If no losses occurred it would be possible to use 10 ml sample and take to dryness before digestion with oxidising acids. The results are shown in Table I. The signal peak heights obtained for 40 ng/10 ml or 4 ppb arsenic show serious and inadvertant losses. The precision for urine with added amounts of arsenic is reasonably good, but 2 ml urine sample gave values ranging between 0.5 and 1.5. Although it is not bad considering that the detection limits are

...6

reached, better precision has been obtained in experiments where no prior drying of urine samples was performed. The samples and standards were placed in the drying oven at 150°C until dry. Although drying in presence of magnesium oxide[3] may have overcome such losses, this work was abandoned in favour of much simpler wet ashing of 2 ml sample volumes. The sample peaks obtained with 2 ml samples were more than adequate in terms of signal/background noise ratio to satisfy the client groups' requirement of a lower reportable limit of 0.1 ppm arsenic.

Table II contains the results obtained with 1 ml urine samples. The duplicates are close and recovery is satisfactory. The results were calculated on the valid assumption that the standard curve is linear up to 20 ng As/ml. The digestions were carried out with 2 ml of fuming nitric acid. The digestates were clear and colourless with no residues or sediment. The detection limit was just about reached in these two samples because the signals were about twice the size of baseline noise. It was concluded, therefore, that detection limit for arsenic by this procedure using 1 ml sample was about 4 ng/ml or 4 ug/l. It was also concluded that lowering the sample volume below 2 ml did not offer any further advantage. Therefore, 2 ml was chosen as the optimum sample volume.

...7

Fuming nitric acid and nitric acid are oxidising agents, but they are not as powerful as a mixture of nitric and perchloric acids. The latter have been used by other workers in the field. The perchloric acid was initially avoided because it was feared that potassium perchlorate might precipitate and carry arsenic with it. This was not found to be the case. There was no precipitate visible. Besides the presence of perchloric acid gives more effective oxidation as well as an indication of the expulsion of excess nitric acid as the first signs of white fumes appear in the test tubes. Table III shows the comparison of results obtained by the use of fuming nitric acid and nitric acid. The former gave accurate results on the urine samples. The sample peaks were somewhat erratic with nitric acid. The standards containing 2, 4 and 6 ng As/ml were also carried along with the samples. It was noticed that unlike perchloric nitric acid digestion different tubes left variable amounts of excess acids at the end of digestion. The digestion of urine with nitric acid did not appear to be complete. The proteins in urine do not oxidise completely with nitric acid alone. Even fuming nitric acid may not be entirely satisfactory because above a certain temperature the NO_2 fumes are lost.

Table IV contains the results obtained with perchloric:nitric acid digestion (1:4 v/v). Two ml

...8

sample and 2 ml digestion mixture were used as per procedure. The results are quite satisfactory. The precision is good. The results calculated by single point calibration and by standard addtion method are 2.45 and 2.53 ng As/ml. Figure 2 shows a sample of typical tracings obtained. The relative standard deviation of the method is 2.5% at 2.5 ug/l arsenic concentration in urine.

A similar experiment was performed using one ml and 2 ml digestion mixture on similar sets of samples and standards. Although the results compared reasonable well, some standards gave lower yield. Therefore, 2 ml was chosen as the optimum volume of acid.

No organically bound arsenic compound was available for recovery experiments, although the importance of such a study was recognized.

Considering the limited time assigned for investigating the method, the results appear to be quite encouraging.

Larger sample volumes may be used if lower detection limits are desired or if very high accuracy is required at concentrations less than 10 ug/l. This will necessitate the use of pre-drying and ashing approaches used by J. G. Lamberton et al[3] and cross references cited. The majority of the lower results reported by them are scattered around an average value of 0.03 ppm arsenic

...9

compared with 0.004 obtained by us. Assuming these samples to be normal urines, our results are an order of magnitude lower. Dr. N. Smith admits that our values agree in general, with those reported in the literature by others. The list of results reported to the Ministry of Health personnel as of the date of this report are appended. The samples were collected by the Ministry of Health personnel. The collection vessels used were prewashed 2 oz. polyethylene bottles. No preservatives were used. There was a degree of dissatisfaction with the storage and shipping of samples before analysis. Recommendations were not closely followed on account of certain unavoidable difficulties on their part. Dr. Smith and her predecessor Dr. Mueller were advised from time to time about these problems through written memos. Nearly all the samples, spikes and duplicates were prepared for analysis by the Health Laboratories staff under the supervision of Mr. Reynold. The samples were not part of a 24 hour collection as is the practice in clinical chemistry field. This fact should be taken into consideration when comparing results with those of other workers in the field.

REFERENCES:

1. Vijan, P. N., and Wood, G. R., Atom. Absorption Newsletter 1974, 13, 33

2. Vijan, P. N., Rayner, A. C., Sturgis, D. S., and Wood, G. R., Analytical Chim. Acta, 1976, 82, 329

3. Lamberton, J. G., Arbogast, B. L., Deinzer, M. L., and Norris, L. A., American Industrial Hygiene Assocn. Journal, July 1972, pages 418-422

TABLE I

EFFECT OF OVEN DRYING ON THE DETERMINATION OF As IN URINE

(SAMPLES DIGESTED WITH 2 ML OF FUMING NITRIC ACID)

SAMPLE	SIGNAL (Peak Height)
Urine (2 ml)	1.5, 1.0, 1.5, 1.0, 0.5, 1.0
Urine + 40 ng As	14.5, 15.0, 15.1, 15.8, 15.0, 15.7
40 ng As only	6.5, 11.6, 13.8

TABLE II

ARSENIC URINE USING 1 ML SAMPLE + 2 ML FUMING NITRIC ACID

Urine Sample	Signal Chart Divisions	ng As/ml	% Recovered	ug As/L (mean)
GW	2	0.4		4
GW	2	0.4		
GW + 100 ug	51.1	11.2	108	
GW + 100 ug	51.1	11.2	108	
EW	1.5	0.3		2.5
EW	1.0	0.2		
EW + 1W ug	49.0	10.8	106	
EW + 100 ug	51.8	11.4	112	
100 ug As	46.0	-		
200 ug As	89.0	-		
BK	00	-		

TABLE III

COMPARISON OF REPRODUCIBILITY AND RECOVERY OF As IN URINE USING FUMING NITRIC ACID AND NITRIC ACID DIGESTION

SAMPLE	ug As/L FUMING HNO_3 (2 ml)	ug As/L NITRIC ACID (2 ml)
Urine (2 ml)	0.5, 0.6, 0.7, 0.6, 0.7 Mean = 0.6 SD = RSD =	1.0, 1.1, 1.2, 1.1, 1.5 Mean = 1.2 SD = RSD =
Urine + 20 ng As	2.7, 2.5, 2.5, 2.9, 2.7, 2.6 Mean = 2.6 SD = RSD =	3.0, 2.8, 3.2, 3.1, 3.0 Mean = SD = RSD =
Urine + 40 ng As	4.6, 4.6, 4.8, 4.7, 4.8, 4.8 Mean = 4.7 SD = RSD =	4.8, 4.8, 4.5, 4.8, 4.7, 5.3 Mean = 4.8 SD = RSD =

TABLE IV

PRECISION & ACCURACY OF As MEASUREMENT IN URINE
(SAMPLES DIGESTED WITH 2 ML OF 4:1 HNO_3-$HClO_4$)

SAMPLE	SIGNAL Peak Height	MEAN Peak Height	RSD	ug As/l*	% Recovery
Urine (2ml)	2.0, 2.0, 2.0 2.0, 2.0, 3.0	2.2	-	2.45	
Urine + 40 ng As	20.2, 18.7, 19.5 20.2, 20.0, 19.0 19.6, 20.0, 19.6	19.6	2.5%	-	97.5
40 ng As only	18.4, 17.9, 17.5	17.9	-	-	

* The value calculated by standard addition technique is 2.53

APPENDIX

TEST RESULTS FOR ARSENIC IN URINE

SAMPLE NUMBER	4	5	6	8	9	12	14	16	18	19	20
ug/L As	<3	<3	6	10	4	17	5	9	9	12	5
SAMPLE NUMBER	21	26	29	30	31	32	33	34	35	38	40
ug/L As	5	5	9	5	5	8	8	5	8	3	9
SAMPLE NUMBER	44	45	47	49	50	52	55	60	62	63	64
ug/L As	8	4	6	12	4	6	9	<3	11	<3	<3
SAMPLE NUMBER	66	67	68	69	70	71	72	73	75	76	77
ug/L As	8	4	4	<3	16	5	4	4	20	9	<3
SAMPLE NUMBER	78	79	80	81	84	85	86	87	88	89	90
ug/L As	4	<3	8	7	7	6	6	8	4	11	5
SAMPLE NUMBER	91	92	93	97	98	99	100	101	102	103	104
ug/L As	17	94	15	10	8	32	4	15	28	5	5
SAMPLE NUMBER	108	110	113	117	118	120	121	122	125	130	132
ug/L As	10	25	10	8	3	3	7	23	7	7	20
SAMPLE NUMBER	141	1	2	3	4	5	6	7	8	9	10
ug/L As	10	6	6	16	10	10	4	15	8	12	8
SAMPLE NUMBER	11	12	13	14	15	16	17	18	19	20	21
ug/L As	8	5	3	14	9	7	7	20	3	3	<3

TEST RESULTS FOR ARSENIC IN URINE

SAMPLE NUMBER	22	23	24	25	26	27	28	29	30	31
ug/L Arsenic	46	7	14	13	8	5	27	16	5	5
SAMPLE NUMBER	32	33	34	35	36	37	38	39	40	41
ug/L Arsenic	11	10	8	4	3	4	<3	3	12	11
SAMPLE NUMBER	42	43	44	45	46	47	48	49	50	51
ug/L Arsenic	10	<3	10	4	4	<3	3	9	4	9
SAMPLE NUMBER	52	53	54	55	56	57	58	59	60	61
ug/L Arsenic	<3	6	<3	5	3	4	10	4	8	7
SAMPLE NUMBER	62	63	64	65	66	67	68	69	70	71
ug/L Arsenic	8	3	8	10	7	4	4	13	11	4
SAMPLE NUMBER	72	73	74	75	76	77	78	79	80	81
ug/L Arsenic	<3	<3	<3	3	<3	4	4	5	4	4
SAMPLE NUMBER	82	83	84	85	86	87	88	89	90	91
ug/L Arsenic	8	4	<3	4	4	10	10	M*	4	M*
SAMPLE NUMBER	92	93	94	95	96	97	98	99	100	101
ug/L Arsenic	4	4	4	<3	7	9	7	5	19	<3
SAMPLE NUMBER	102	103	104	105	106	107	108	109	110	111
ug/L Arsenic	4	<3	<3	<3	38	13	4	8	9	36

M* - Missing

TEST RESULTS FOR ARSENIC IN URINE

SAMPLE NUMBER	112	113	114	115	89	116	117	118	119	120
ug/L Arsenic	11	6	8	13	4	4	4	<3	4	7

SAMPLE NUMBER	121	122	123	124	125	126	127	128	129	130
ug/L Arsenic	<3	4	4	<3	9	6	7	6	4	4

SAMPLE NUMBER	131	132	133	134	135	136	137	138	139	140
ug/L Arsenic	<3	<3	<3	17	<3	13	55	12	94	25

SAMPLE NUMBER	141	142	143	144	151	152	153	154	155	156
ug/L Arsenic	4	11	12	5	10	<3	6	4	6	10

SAMPLE NUMBER	157	159	41	R	B	1	2	3	4	5
ug/L Arsenic	6	12	11	10	10	9.0	9.0	6.9	4.4	4.4

SAMPLE NUMBER	6	7	8	11	12	13	14	15	16	17
ug/L Arsenic	19.4	15.0	4.4	3	6.4	-	6.2	16.2	8.9	3

SAMPLE NUMBER	18	19	20	101	102	103	104	106	107	108
ug/L Arsenic	6.3	8.2	8.2	57.5	11.3	3	-	13.8	9.4	3

SAMPLE NUMBER	109	110	111	113	-
ug/L Arsenic	6.2	9.4	6.3	16.2	20.6

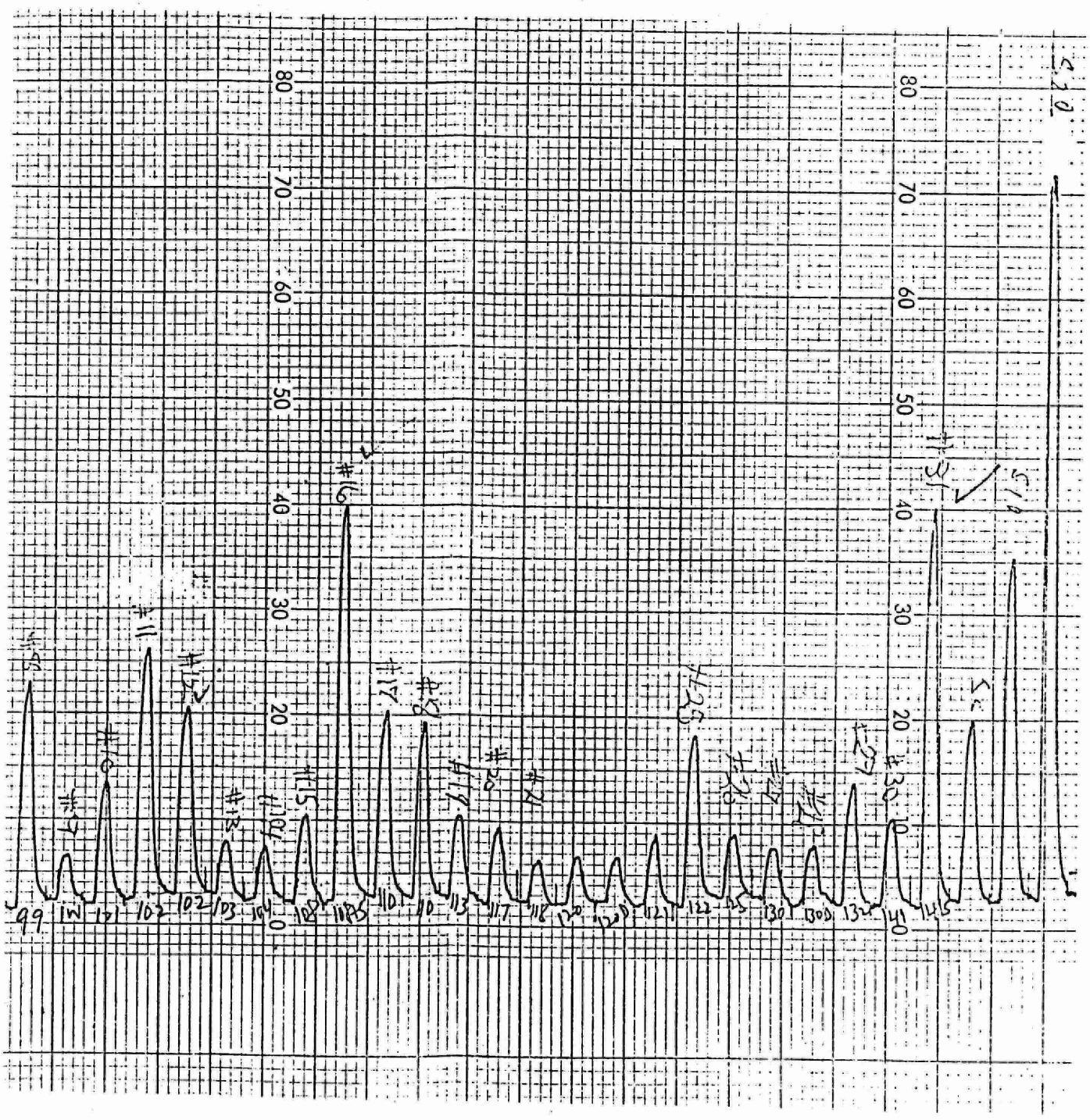
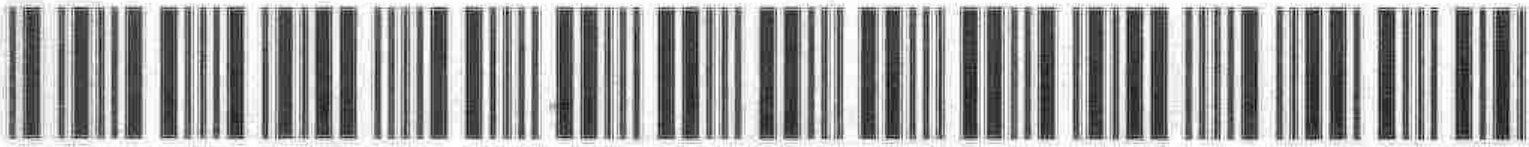


FIG. 2 TYPICAL RECORDER TRACINGS


9693600009325